CHAPTER 2

HEAT SHOCK PROTEINS, UNFOLDED PROTEIN RESPONSE CHAPERONES AND ALZHEIMER'S DISEASE

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Abstract: Molecular chaperones interact with cellular proteins to ensure proper folding and transport between or into organelles. They also associate with mature proteins that have unfolded (and become prone to aggregation) because of an environmental insult such as heat shock. There is a large body of evidence that protein quality control mechanisms involving the HSP family of molecular chaperones, as well as proteasomal and lysosomal functions, become impaired with aging and contribute to a variety of neurodegenerative diseases. Promising therapeutic approaches tested in animal models of Parkinson's and polyglutamine diseases include the up-regulation of molecular chaperones to prevent protein misfolding and aggregation and to facilitate clearance mechanisms. In spite of a slow start, the role of molecular chaperones in Alzheimer's disease is increasingly being elucidated at the molecular level. This chapter summarizes the nature of the cellular stress response that is induced in Alzheimer's disease and examines current research related to the function of molecular chaperones in the cellular metabolism of tau and β-amyloid peptide

Keywords: β-amyloid; tau; Alzheimer's disease; neurodegeneration; heat shock proteins; protein misfolding

HEAT SHOCK PROTEINS, THE AGING PROCESS AND NEURODEGENERATION

Sophisticated quality control mechanisms are required to ensure the proper synthesis, folding, post-translational modifications, and transport of proteins within a highly crowded macro-molecular, intracellular environment that favors protein

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misfolding and aggregation (Ellis, 2001). Over the past few years, considerable attention has been focused on a highly conserved family of proteins, termed chaperones, which helps to fold proteins into their native conformations. Historically, chaperones are referred to as heat shock proteins (Hsp), if they respond to heat shock, or glucose regulated proteins (Grp), if they respond to metabolic stress, such as glucose insufficiency. Hsp are generally cytoplasmic, while the corresponding Grp are components of the unfolded protein response (UPR) of the endoplasmic reticulum and Golgi.

Protein misfolding results in the exposure of hydrophobic domains normally buried in the interior of the native structure. Interactions between these exposed hydrophobic regions lead to the formation of toxic aggregates, which include oligomers, protofibrils and fibrillar deposits having the chemical signature of amyloid. Hsp not only detect and refold denatured proteins, but are also actively involved in the triage of unsalvageable products to the major cellular protein degradation system, the proteasome. Thus, Hsp in the role of chaperones are key components of the machinery that maintains a delicate balance between natively folded functional proteins and aggregation-prone misfolded proteins. The latter may form acutely to some cell stressors or build-up over a lifetime and lead to cell death.

The accumulation of misfolded proteins is one hallmark of aging. During the life-span of a stable protein, various post-translational modifications occur (Harding et al., 1989). In some cases, for instance oxidative damage, these modifications induce conformational changes that impair protein function, and cannot be reversed by Hsp. Therefore, the only solution to protect the cell from these misfolded proteins is their elimination or sequestration. Aging is accompanied by decreases in proteasome activity (Conconi et al., 1996) as well as autophagic lysosomal protein degradation or "autophagy" (Cuervo and Dice, 2000). When the chaperone-degradation system fails at any of several steps, abnormal proteins accumulate as aggregates or inclusions. The problem may also be amplified when Hsp and other protective chaperones get trapped over time in these insoluble inclusions, therefore reducing their cellular levels and leaving the cell more susceptible to further stresses. Indeed, as cells age they also lose their ability to fully activate the unfolded protein stress response or UPR as defined below (Liu et al., 1989; Fargnoli et al., 1990; Sherman and Goldberg, 2001). Thus, aging rats display reduced levels of endoplasmic reticulum chaperones, increased ubiquitination and impaired activation of the stress response in the hippocampus (Paz Gavilan et al., 2006).

Dysfunction of the protein folding and degradation machinery is also believed to contribute to a variety of human diseases (Cummings et al., 1998; Lam et al., 2000; Bence et al., 2001; morley and moromoto, 2004; Lindsten et al., 2002). Thus, subtle impairments in the chaperone system that may occur with aging, together with increases in abnormally folded client protein expression or production, may result in aberrant accumulation and aggregation of cytotoxic proteins and neurodegeneration (Cohen et al., 2006). Increasing evidence points to a critical role for molecular chaperones in neurodegenerative diseases (DeArmond and Prusiner, 1995; Bonini, 2002; Sakahira et al., 2002). Several neurodegenerative

diseases also appear to involve an early impairment of the stress response (Batulan et al., 2003; Magrane et al., 2005), further compromising the function and survivability of neurons. Since they are post-mitotic, dilution of cytotoxic misfolded proteins by cell division is not an option. For instance, it has been recently shown that abnormal S-nitrosylation of protein disulfide isomerase, an endoplasmic reticulum chaperone, is found in brain samples of sporadic Parkinson's and AD cases. The S-nitrosylation modification blocks protein disulfide isomerase protective function that is part of the endoplasmic reticulum stress response (Uehara et al., 2006). In another case of endoplasmic reticulum chaperone dysfunction in neurons, a mutation in the gene *sil1* that encodes a co-chaperone of Grp78, a crucial endoplasmic reticulum chaperone, leads to protein accumulation and neurodegeneration (Zhao et al., 2005). Importantly, mutations that compromise the activity of Hsp family members lead to several rare syndromes, such as hereditary spastic paraplegia (mitochondrial Hsp60), desmin-related myopathy (αB-crystallin), Marinesco-Sjogren syndrome (Sil1), axonal Charcot-Marie-Tooth disease and distal hereditary motor neuropathy (Hsp27), and distal motor neuropathy (Hsp22) (Vicart et al., 1998; Hansen et al., 2002; Evgrafov et al., 2004; Irobi et al., 2004; Anttonen et al., 2005; Senderek et al., 2005).

In broad terms, most of the neurodegenerative diseases can be considered as disorders of protein misfolding, referred as "foldopathies" (Kosik and Shimura, 2005). Several age-related disorders such as AD, Parkinson's disease, amyotrophic lateral sclerosis, prion diseases and the polyglutamine expansion diseases share a common pathology related to protein misfolding and progressive intracellular accumulation of specific but unrelated toxic proteins that target select cell populations. Each protein abnormality also triggers a different cellular response within the protein folding machinery and the degradation pathways. In this chapter, we will review the nature of this stress with respect to AD and examine some recent studies related to the function of heat shock and stress related proteins in the cellular metabolism of tau and β-amyloid peptide.

APP PROCESSING AND INTRACELLULAR EVENTS IN ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is the most common neurodegenerative disorder and the leading cause of dementia. It is usually diagnosed through recognition of a restricted impairment in memory that then expands to involve other cognitive process. AD is associated with a characteristic combination of morphological brain alterations and most often arises sporadically from the combination of genetic risk factors and unknown environmental/ aging processes (sporadic AD) or much less often (2%) directly from heritable defects in key proteins (familial AD, FAD). While mutations in either the amyloid precursor protein (*APP*), presenilin-1 (*PS1*) or presenilin-2 (*PS2*) genes cause the majority of early-onset FAD, the molecular basis for the later onset sporadic forms of AD remains largely unknown. However, evidence implicates oxidative stress (Markesbery, 1997) and cardiovascular risk factors

(de la Torre, 2002), as well as from the increased incidence of AD in individuals possessing specific apolipoprotein E (APOE4) or sortilin-related receptor (SORL1) genotypes (Cedazo-Minguez and Cowburn, 2001; Rogaeva et al., 2007).

The major pathological hallmarks in the brains of AD patients are extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs). However, amyloid angiopathy and inflammatory changes also occur in most cases. Amyloid deposits are derived from the amorphous aggregation of a number of proteins, of which a small peptide referred to as β-amyloid (Aβ) is the main component. The other observable pathological structures, NFTs, are paired helical filaments derived from the aggregation of hyperphosphorylated forms of the microtubule-associated protein tau (τ). It is now widely accepted that Aβ peptide has a primary role in the development of AD (Hardy and Allsop, 1991; Hardy and Selkoe, 2002; Wirths et al., 2004). However, in the order of events leading to AD, the exact roles played by intracellular Aβ and τ are still to be elucidated. Nevertheless, increasing evidence both from transgenic mice expressing familial AD mutations and human AD patients supports an early role for intracellular Aβ accumulation (LaFerla et al., 2007). Indeed, although the formation of NFTs closely parallels the progression and anatomic distribution of neuronal loss in AD, intraneuronal Aβ accumulation precedes the deposition of amyloid plaques and the appearance of NFTs, and correlates with the first indications of cognitive deficits (Gouras et al., 2000; D'Andrea et al., 2001; Wirths et al., 2001; Oddo et al., 2003; Billings et al., 2005; Oakley et al., 2006; Knobloch et al., 2007). It is worth mentioning that intracellular Aβ has also been clearly associated with Down's syndrome and the human muscle disease inclusion body myopathy (Tseng et al., 2004). Several observations also suggest that both intracellular amyloid and τ pathologies may be causally linked (Blurton-Jones and Laferla, 2006). Recent studies have shown that the accumulation of intracellular Aβ in vivo inhibits the proteasome, and that proteasome impairment leads to the buildup of abnormally phosphorylated τ protein (Oddo et al., 2004; Tseng et al., 2007).

The Aβ peptide is generated by endoproteolysis of the APP, a single pass, type I membrane protein. Three different groups of enzymes, termed α-, β-, γ-secretases, sequentially cleave APP in two alternate processing pathways. In the most common non-amyloidogenic pathway, membrane proximal cleavage by α-secretases (at a position 83 amino acids away from the carboxy-terminus of APP) precludes generation of Aβ peptide. A large amino-terminal domain (sAPPα) is secreted into the extracellular medium, and the resulting C83 fragment is then cleaved by a γ-secretase complex cleavage (composed of presenilin 1 or 2, nicastrin, anterior pharynx defective and presenilin enhancer 2) to generate a short fragment named p3. In the amyloidogenic pathway, cleavage of APP by β-site APP cleaving enzyme 1 (BACE1; at an extracellular position 99 amino acids away from the C-terminus of APP) results in the release of sAPPβ into the extracellular space, and subsequent cleavage of the resulting C99 fragment by the γ-secretase complex results in the generation of Aβ peptide. Processing of APP by β-secretase occurs in endosomes following cell surface receptor recycling, but can also occur in the endoplasmic

reticulum/trans-Golgi network, prior to APP secretion, or at the plasma membrane (Hardy and Selkoe, 2002; Kins et al., 2006; Vetrivel and Thinakaran, 2006). Thereby, generation of Aβ peptide is likely to occur in several intracellular compartments.

Several Aβ variants have been described which differ in their length. The most abundant Aβ peptide is 40 residues long (Aβ40), whereas a small proportion is 42 residues long (Aβ42). This longer variant is more hydrophobic and prone to aggregation, and is the predominant form found both intracellularly and in cerebral plaques (Gouras et al., 2000). The Aβ40 isoform is predominant in congophilic angiopathy. Aβ can be found in different assembly states (monomers, oligomers, protofibrils and fibrils), which have important physiological and pathological effects. While monomeric Aβ appears to be the less neurotoxic species, oligomers and protofibrils are the most pathological forms (Walsh et al., 2002; Cleary et al., 2005; Lesne et al., 2006). Similar conclusions were drawn in a number of other neurodegenerative diseases (Caughey and Lansbury, 2003). Aβ oligomerization first occurs intraneuronally, when associated with synaptic pathology (Walsh et al., 2000; Takahashi et al., 2004; Oddo et al., 2006). Although not clear yet, fibril formation appears to result from β sheet formation and linear cross-stacking, since the Aβ42 peptide contains exposed hydrophobic domains that can seed abnormal protein aggregation (Bitan et al., 2003; Kayed et al., 2003).

Consistent with clinical observations on the importance of intracellular accumulation, we and others have reported on the toxic effects of intraneuronal Aβ accumulation in model systems (LaFerla et al., 1995; Zhang et al., 2002; Magrane et al., 2004). Observations made when using synthetic Aβ peptides applied to cell cultures do not necessarily reflect what happens intracellularly when Aβ accumulates (Zhang et al., 2003; Magrane et al., 2005). Accumulation of intracellular Aβ42 may affect a variety of signal transduction pathways including Akt and MAPK family members that have important roles in neuronal function (Yuan and Yankner, 2000). We previously reported that intracellular Aβ42 deposition disrupts signaling through the Akt pathway, both in vitro and in vivo (Magrane et al., 2005). Others observed that the MAPK pathway may also be affected in an in vivo model of intracellular Aβ accumulation (Echeverria et al., 2004). We first described that the down-regulation of the Akt survival pathway caused a suppression of the stress response (Magrane et al., 2005). Moreover, when τ becomes abnormally phosphorylated, it aggregates and loses its ability to maintain stability of the axonal microtubules, which are the conduits for intracellular protein traffic (Mandelkow et al., 1995). Thus, both aberrant processing of APP and/or post-translational modifications affecting τ, generate species susceptible to aggregation and shown to be neurotoxins. Several other common neurodegenerative diseases have pathological features similar to AD, all characterized by inclusions of misfolded proteins. Indeed, it has been shown that soluble oligomers from Alzheimer's, Parkinson's, polyglutamine and prion diseases share a common structural feature that is recognized by a single antibody, an observation that points to the highly related nature of these diseases (Kayed et al., 2003).

While many mechanisms have been suggested to explain the starting point of AD pathogenesis, it is clear that neurons first fail in function and then die for lack of ability to buffer multiple metabolic stresses arising from overproduction and/or failure to clear neurotoxic amyloidogenic proteins. In the sequence of intraneuronal events that lead to AD pathogenesis, alterations in APP processing, Aβ turn-over and τ phosphorylation factor early in the disease progression. One promising role for Hsp in this process is to hasten Aβ removal. When clearance mechanisms become overwhelmed, Aβ oligomers eventually form insoluble fibrils that are deposited as amorphous inclusions that can include Hsp (Muchowski and Wacker, 2005). Hsp have been shown to accumulate in senile plaques and to be up-regulated in AD brain (Hamos et al., 1991; Perez et al., 1991; Yoo et al., 2001; Sahara et al., 2005). While several members of the Hsp family are shown to interact with key players of AD pathogenesis, not many studies have explored the role of the stress response in AD. Most of them have focused on abnormal τ phosphorylation and not until recently, has the role of heat shock and stress proteins in Aβ accumulation been addressed at the molecular level.

THE UPR AND CYTOPLASMIC CHAPERONE RESPONSES TO β-AMYLOIDOGENESIS

All proteins destined to the plasma membrane, including APP, first translocate from ribosomes into the endoplasmic reticulum(ER), where a group of chaperones work together to ensure the proper folding and assembly of nascent proteins, so that trafficking towards the secretory and the endocytic/lysosomal pathways can proceed. These chaperones include: BiP/Grp78 (glucose-regulated protein 78), Grp94, Grp170/ORP150, Grp58/ERp57, peptidyl prolyl isomerase, ERp72, calnexin, calreticulin, EDEM, Herp, protein disulfide isomerase and co-chaperones Sil1 and p58IPK. When misfolding and aggregation occurs in the endoplasmic reticulum, a specific ER stress response, known as the unfolded protein response (UPR) is activated. First, the response is initiated by the dissociation of BiP/Grp78 from unfolded protein "stress sensors" PERK (pancreatic ER serine/threonine kinase) and Ire-1. Then, expression of endoplasmic reticulum-resident chaperones (such as Grp78 and Grp94) is increased. Additionally, general protein synthesis is attenuated by translational shut-down. A third arm of the stress response is the activation of an endoplasmic reticulum-associated degradation (ERAD) pathway, by which misfolded proteins are retrotranslocated into the cytosol for ubiquitination and degradation (Bonifacino and Weissman, 1998; Ellgaard et al., 1999; McCracken and Brodsky, 2003). When the problem persists, C/EBP homologous protein (CHOP) and other factors are activated to induce apoptosis (Rao et al., 2004).

Experimental evidence points to endoplasmic reticulum being an important cellular compartment in which Aβ generation can occur (Hartmann et al., 1997; Wild-Bode et al., 1997; Tomita et al., 1998; Greenfield et al., 1999). Moreover, endoplasmic reticulum-resident chaperones interact with APP and APP proteolytic fragments (Yang et al., 1998b; Fonte et al., 2002; Hoshino et al., 2007) and thereby

could affect the generation of Aβ. It has been suggested that mutations in the *PS1* or *PS2* genes contribute, in part, to neuronal vulnerability through down-regulation of the UPR signaling pathway and impaired Grp78 induction (Guo et al., 1999; Katayama et al., 1999, 2001, 2004; Niwa et al., 1999). Of note, not all studies show such changes (Sato et al., 2001). This would have the undesirable effect of impairing the neuron's ER-based capability to prevent the accumulation of toxic proteins. The phenomenon of decreased ER chaperone expression in familial AD brain, (e.g. Grp78 levels (Kudo et al., 2002)), resembles what has been proposed to occur during aging (Sherman and Goldberg, 2001). In sporadic AD brains on the other hand, increased expression of proteins of the UPR has been observed in neurons without signs of neurodegeneration or NFTs (Hamos et al., 1991; Chang et al., 2002; Onuki et al., 2004; Hoozemans et al., 2005). This is in contrast to other studies where a decrease in Grp78 or no changes were observed (Katayama et al., 1999; Sato et al., 2000; Kudo et al., 2002). In another study, BiP/Grp78 is increased in AD brain coincident with down-regulation of cell cycle proteins and G1 phase arrest (Hoozemans et al., 2006). To reconcile these studies, it is plausible that initial activation of the UPR in viable AD neurons can be neuroprotective, while sustained activation lead to failure and heralds neurodegeneration (Ghribi et al., 2001; Chen et al., 2004; Tessitore et al., 2004; Brewster et al., 2006). Involvement of the UPR has been demonstrated in other neurodegenerative conditions such as juvenile Parkinson's disease and in Pelizaeus-Merzbacher disease (Imai et al., 2001; Southwood et al., 2002).

After the discovery that secretases are present in different compartments of the secretory and the endosomal/lysosomal pathways, and that APP C-terminus bearing fragments (CTF) and Aβ are generated intracellularly (Busciglio et al., 1993; Wertkin et al., 1993; Cook et al., 1997; Hartmann et al., 1997; Skovronsky et al., 1998), attention was put on potential ER quality control mechanisms that could alter APP processing and Aβ production/removal. First, interactions between APP and chaperones were revealed in the endoplasmic reticulum, where APP folding and maturation occurs. Therein, holoAPP directly interacts with Grp78, a resident chaperone that transiently associates with normally maturing polypeptides and more stably with misfolded or incompletely assembled proteins. When Grp78 is over-expressed, APP translocation from the endoplasmic reticulum to the Golgi is inhibited, APP maturation fails, and the levels of CTF and Aβ released into the medium decrease (Yang et al., 1998b; Kudo et al., 2006; Hoshino et al., 2007). Grp78 protects against excitotoxic and amyloid cell death (Yu et al., 1999). Next, the over-expression of certain other chaperones in the endoplasmic reticulum, have similar activities on APP processing. Thus, Grp170/ORP150 (oxygen-regulated protein 150) decreased the levels of both Aβ40 and 42 released into the medium, whereas calnexin decreased the release of only Aβ42 (Hoshino et al., 2007). Transient interaction with calreticulin, which is involved in the maturation of glycoproteins in the secretory pathway, is also required for APP trafficking and maturation through the endoplasmic reticulum and early cis-Golgi. It is known that holoAPP/calreticulin complex formation requires both prior binding

to Grp78 and N-glycosylation to occur (Johnson et al., 2001). Despite this interaction, calreticulin does not affect Aβ release into the medium (Hoshino et al., 2007). Although it is unclear how these different effects on wild type APP processing may occur, one possible explanation is that sublocalization of the various ER chaperones leads to a differential ability to activate the ERAD pathway. The data also suggests that expression of mutant APP, and in particular Aβ production, activates the UPR (Hoshino et al., 2007), although the role of intracellular Aβ accumulation was not explored. Induction of certain endoplasmic reticulum chaperones could therefore be therapeutically beneficial for the treatment of AD.

We turn attention now onto the cytoplasmic chaperone system. On the cytosolic site of the endoplasmic reticulum, APP has been shown to interact with heat shock cognate (Hsc) 73 (Kouchi et al., 1999). Although the significance of this interaction remains unclear, it presumably facilitates APP ubiquitination and degradation. In fact, the proteasome has been shown to be involved in the catabolism of APP and its secretase cleavage products (Marambaud et al., 1997; da Costa et al., 1999; Nunan et al., 2001; Flood et al., 2005; Kumar et al., 2007). This is reminiscent of the increase in Hsc/Hsp70 bound to polyubiquitinated hyperphosphorylated τ that was observed in the presence of proteasome inhibitors (Petrucelli et al., 2004; Shimura et al., 2004b). Moreover, CHIP (carboxy terminus of the Hsc70-interacting protein) over-expression increases cellular APP levels and promotes both APP and phospho-τ ubiquitinations (Petrucelli et al., 2004; Shimura et al., 2004b; Kumar et al., 2007) in accordance to a proposed role of CHIP to act as a molecular triage center (Connell et al., 2001).

Aβ-targeted to the secretory pathway was found to activate the cytosolic stress response and to interact with cytosolic signal and chaperone proteins (Suhara et al., 2003; Magrane et al., 2004, 2005; Zhang et al., 2004; Kumar et al., 2007). A stress response involving up-regulation of Hsp70 levels in AD and Down's syndrome temporal cortex confirms the in vitro data (Yoo et al., 1999). It is unclear how Aβ exits the endoplasmic reticulum, although reverse translocation through the ERAD pathway is a likely explanation. Aβ may also gain access to the cytosol due to leakage from lysosomes (Yang et al., 1998a; McCracken and Brodsky, 2003). Cytosolic Aβ has been shown to be highly cytotoxic to primary neurons (Zhang et al., 2002), and Aβ expressed intracellularly in an in vivo model of AD was found to interact directly with HSP70 family members (Fonte et al., 2002). Since Hsp70 has roles in preventing protein aggregation and promoting protein degradation (Muchowski et al., 2000; Dul et al., 2001; Chan et al., 2002; Dou et al., 2003), it is plausible that Hsp70 is critical in the proteasomal handling or in the sequestration of intraneuronal Aβ. Interestingly, over-expression of some Hsp reduced intracellular Aβ levels and, consequently, Aβ-induced neuronal death (Kumar et al., 2007). Other mechanisms involving loss of Hsp function in AD are apparent. For instance, mortalin (mtHsp70/Grp75) is a heat non-inducible mitochondrial protein that when oxidatively damaged in AD, results in reduced mitochondrial import of essential proteins (Yaguchi et al., 2007).

REMOVAL OF HYPERPHOSPHORYLATED tau (τ)

Tau (τ) is normally a highly soluble and natively unfolded protein that undergoes continuous turnover in neurons (Dickey et al., 2006). It remains unclear how the quality control system is able to distinguish between normal phospho-τ and aberrantly hyperphosphorylated τ species, although it has been demonstrated that early in AD pathogenesis, a combination of abnormal phosphorylation and certain conformational changes to τ serves as the misfolding event that is recognized by the chaperone machinery (Weaver et al., 2000; Ghoshal et al., 2001). Hyperphosphorylated τ is toxic to neurons in vitro and in vivo and has been clearly implicated in AD progression (Gomez-Isla et al., 1997; Gamblin et al., 2003; Kobayashi et al., 2003; Roberson et al., 2007). The mechanisms underlying τ-mediated neurotoxicity however remain unclear. Those isoforms phosphorylated by the kinases glycogen synthase kinase 3beta (GSK3-β) and Cdk5 (Lucas et al., 2001; Cruz et al., 2003; Noble et al., 2003) are particularly suspect. Many of these sites are dephosphorylated by PP2A, and a deficiency of phosphatase activity has also been implicated in τ hyperphosphorylation and impairment of behavior performance in rats (Sun et al., 2003). As is the case with Aβ entities, soluble τ oligomers and/or protofibrils probably mediate τ-associated neurodegeneration (Dickey et al., 2006). To reduce phosphorylated τ concentrations and τ-associated cellular toxicity, a variety of protective mechanisms involving the stress response are activated. They include binding of abnormal τ to Hsp70 to prevent toxic conformations of the protein, ubiquitination of τ for degradation by the proteasome, segregation of τ aggregates from the cellular machinery, and recruitment of anti-apoptotic molecules. Thus one mechanism for τ accumulation may be insufficient Hsp-mediated phospho-τ ubiquitination and degradation.

Several studies have explored the role of Hsp in hyperphospho-τ degradation, and focused on a complex comprised of Hsp70/Hsc70, Hsp90 and the E3 ubiquitin ligase CHIP (carboxy terminus of Hsc70-interacting protein). The Hsp/CHIP complex is a highly sensitive and tightly regulated quality control mechanism, involving multiple players that may also compete for the refolding or degradation of the abnormal protein (Johnson et al., 1998; Grenert et al., 1999; Liou et al., 2003). CHIP works together with BAG-1, an ubiquitin domain co-chaperone protein that accepts substrates from Hsc/Hsp70 and presents them to the CHIP-ubiquitin conjugation machinery and onto the proteasome (Luders et al., 2000; Demand et al., 2001; Qian et al., 2006). The recognition of τ by Hsc70 and Hsp90 suggests that phosphorylation may serve to disrupt the native structure of τ, targeting it for processing by the Hsc70/CHIP complex. The current view in the field is that if Hsc/Hsp70 system is unable to restore proper folding of τ, then the ubiquitin domain protein BAG-1 and the ubiquitin ligase CHIP, in collaboration with the E2 conjugating enzyme UbcH5B, can shift the chaperone activity of Hsc/Hsp70 from protein folding to an assist role in degradation (Petrucelli et al., 2004; Shimura et al., 2004b; Dickey et al., 2007). CHIP appears to ubiquitinate phosphorylated τ, not only to promote its proteasomal degradation but also to sequester it into insoluble filamentous aggregates and in so doing prevent cell death (Shimura et al., 2004b; Dickey et al., 2006).

Correspondingly, deletion of CHIP in mice results in accumulation of soluble phosphorylated τ in the brain (Dickey et al., 2006). CHIP also acts as a stress sensor (Qian et al., 2006) to positively regulate heat shock factor (HSF)-1 activity (Dai et al., 2003) and to terminate HSP70 through degradation when misfolded protein levels are returned to acceptable levels. HSF1 is a key transcriptional factor that controls the levels of the constituents of the cellular stress response.

Induction of a stress response by increasing levels of Hsp70 (and 90) in τ-transfected cell cultures prevents insoluble τ aggregates and τ phosphorylation, increases the solubility of τ and promotes the normal association of τ with microtubules (Dou et al., 2003). The same authors also demonstrated that Hsp70 (and Hsp90) bind τ and that both Hsp levels are lower in the brains of AD patients bearing τ aggregates and in transgenic mice expressing a mutant form of human τ that is responsible for fronto-temporal dementia (Dou et al., 2003). Over-expression of Hsp70 alone in vivo appears to reduce steady state levels and attenuate partitioning of τ into the high molecular weight detergent insoluble fraction (Petrucelli et al., 2004).

Hsp27 has also been shown to bind directly to phosphorylated τ and attenuate its toxicity by facilitating its degradation (through an ubiquitin-independent pathway) and/or dephosphorylation (Shimura et al., 2004a). Hsp27 plays a critical role in neuronal metabolism and survival, and can inhibit caspase activation (Concannon et al., 2003).

Hsp90 is involved in the folding and stabilization of multiple client proteins (Zhao and Houry, 2005). A central role for Hsp90 in the development of AD and associated tauopathies (Dickey et al., 2007) has resulted in the identification of several Hsp90 inhibitors as potential therapeutic tools in neurodegenerative diseases (Dickey et al., 2006; Waza et al., 2006a). Inhibition of Hsp90 by blockade of the refolding pathway promotes degradation of proteins bound to Hsp90 and usually causes the activation of the HSF1. Thus it has been recently reported that inhibition of Hsp90 actually leads to a decrease in phosphorylated τ levels. However, this action proved independent of HSF1 activation. The mechanism is via increased τ turnover and degradation mediated by CHIP (Dickey et al., 2006). Although somewhat alternative to the HSP90 results of Dou et al. presented above, this is reminiscent of the proteasome-dependent reduction in mutant polyglutamine expanded androgen receptor levels by a geldanamycin-like inhibitor of HSP90 (Waza et al., 2006b).

SMALL HSP, CHAPERONINS AND OTHER STRESS-RELATED PROTEINS IN AD

Although the role of other stress-related proteins in AD remains largely unexplored, some advances have been made more recently, specially related to small Hsp (sHsp), chaperonins and Hsp104. The sHsp are a family of chaperones, with subunit molecular masses ranging from 15 to 40 kDa, that bind to exposed hydrophobic residues but lack active refolding capabilities (ATP-independent chaperones). They

recognize proteins in the early stages of denaturation and help to maintain unfolded proteins in a folding-competent state (Lee et al., 1997). Subsequent refolding is thought to occur by Hsp70 and/or chaperonin function. In humans, the sHsp family comprises 10 members, among which αB-crystallin, Hsp27, Hsp20, HspB2 and HspB8 (Kappe et al., 2003).

αB-crystallin is the prototypical sHsp and contains a characteristic highly conserved carboxy-terminus "α-crystallin domain" that defines the members of this sHsp subfamily. αB-crystallin is the main component of the eye lens where it maintains a clear amorphous composition by preventing denatured proteins from aggregating to form opaque inclusions (Horwitz, 2000). Indeed, Aβ has been observed to accumulate in the cataracts of AD patients and to colocalize with αB-crystallin (Goldstein et al., 2003). αB-crystallin is expressed in both neurons and glia of the normal brain, and in various neurodegenerative diseases, including AD (Iwaki et al., 1992; Mao et al., 2001; Yoo et al., 2001). Changes in expression of αB-crystallin and Hsp16, among others, in response to intracellular Aβ42 expression have been observed (Link et al., 1999, 2003). In addition, αB-crystallin was shown to directly interact with Aβ both in cell culture and in a transgenic *C. elegans* model that expresses human Aβ intracellularly (Stege et al., 1999; Liang, 2000; Fonte et al., 2002). As in the case with Hsp70/CHIP, this sHsp associates with ubiquitinated τ (Goldbaum and Richter-Landsberg, 2004). αB-crystallin has been shown to increase the neurotoxicity of Aβ, possibly by preventing its aggregation into insoluble fibrils (Stege et al., 1999; Raman et al., 2005; Narayanan et al., 2006).

Hsp27 is expressed both in normal and AD brains, and appears to be associated with amyloid plaques and NFT (Renkawek et al., 1994; Stege et al., 1999; Wilhelmus et al., 2006a). Increased expression of Hsp27 has been found in AD brains (Renkawek et al., 1993; Stege et al., 1999) and in Dementia with Lewy Bodies (Outeiro et al., 2006). Furthermore, Hsp27 directly interacts with Aβ and inhibits fibril formation in vitro, possibly by interfering with the nucleation process in the early phase of amyloidogenesis (Kudva et al., 1997). In addition, Hsp27 binds to hyperphosphorylated τ and promotes its degradation by a proteasome-independent pathway and, when over-expressed, prevents hyperphosphorylated τ-mediated cell death (Shimura et al., 2004a).

Although direct interaction between Aβ and some sHsp remains to be demonstrated in vivo, and their potential role in intraneuronal Aβ aggregation has not been proved, it has recently shown in vitro that Hsp20, Hsp 27 and αB-crystallin, but not HspB2, bind to Aβ, prevents Aβ aggregation and attenuate toxicity (Lee et al., 2005, 2006; Wilhelmus et al., 2006b). While the usual function of sHsp is in intracellular surveillance, they are also expressed by reactive astrocytes and their cytoprotective role against experimental Aβ toxicity seems to be explained by interference with extracellular oligomerization at the cell surface (Wilhelmus et al., 2006b). Immunohistological studies have found that extracellular HspB2 is strongly expressed in fibrillar amyloid deposits around the cerebral vessels, and that Hsp20 is mainly associated with non-fibrillar Aβ in diffuse senile plaques (Wilhelmus et al., 2006a).

In addition, another member of the sHsp family, HspB8, binds to distinct Aβ species and inhibits the aggregation of mutated huntingtin (Carra et al., 2005; Wilhelmus et al., 2006c). The results suggest that while sHsp may have different affinities for the various Aβ species and other amyloidotic proteins, they have potential to play a significant role in Aβ deposition in AD brains.

Chaperonins (Cpn) are a family of sequence-related proteins of about 60 kDa that are classified in two groups: group I chaperonins are found in bacteria and organelles (mitochondria and chloroplasts), and group II chaperonins are found in the cytosol of eukaryotes and Archaea. The mitochondrial chaperonin Cpn60 is primarily found in the mitochondrial matrix. Both synthetic Aβ peptide treatment and intraneuronal Aβ accumulation reduced levels of mitochondrial Cpn60, without similarly affecting other Hsp levels, such as Hsp70 and Hsp90 (Zamostiano et al., 1999; Veereshwarayya et al., 2006). Mitochondrial Cpn60 overexpression protected components of the electron transport chain and enzymes of the mitochondrial matrix from Aβ expression-induced toxicity, although it had no effect on Aβ levels or oligomerization (Veereshwarayya et al., 2006). Similarly, reduced expression of mitochondrial Cpn60 has been reported in cells derived from individuals with Down's syndrome, which shares a number of characteristic lesions with AD including intraneuronal Aβ accumulation (Bozner et al., 2002). Similarly, cytoplasmic levels of Cpn60, a specific chaperonin for actin and tubulin, also decrease in AD-affected neurons leaving the cytoskeletal proteins deficient and aggregated (Schuller et al., 2001).

Another chaperone-related family of proteins is the class I family of Clp/Hsp100 AAA^+ ATPases, to which Hsp104 belongs. Hsp104 does not associate with any protease or ligase, unlike other Clp1 proteins, and does not protect from denaturation, but it acts as a molecular chaperone to rescue proteins from an aggregate state, with the help of the Hsp70 system (Parsell et al., 1994; Lee et al., 2004). Hsp104 was shown to inhibit both Aβ and α-synuclein aggregate formation in vitro (Kong et al., 2005).

NEUROPROTECTION AND THERAPEUTIC STRATEGIES

Several studies have convincingly demonstrated that increased chaperone expression can suppress the neurotoxicity caused by accumulation of neurotoxic proteins. Interventions that enhance or boost a deficient stress response may have therapeutic value in limiting the neuronal dysfunction and loss that defines neurodegenerative disease states (Rochet, 2007). Thus, Hsp can delay the onset and the outcome of protein-misfolding diseases, such as in transgenic models of Parkinson's and polyglutamine diseases such as spinocerebellar ataxia (Warrick et al., 1999; Chan et al., 2000; Fernandez-Funez et al., 2000; Kazemi-Esfarjani and Benzer, 2000; Cummings et al., 2001; Auluck et al., 2002). Recent findings suggest that Hsp can also be neuroprotective in AD, but this area of research remains largely unexplored having been focused on in vitro and cell culture studies. The lack of

suitable animal models that accurately replicate the characteristics of the human disease and the complex etiology of AD are possible reasons for this state. Hsp have been shown to modulate the aggregation of Aβ peptide in a cell-free system (Evans et al., 2006; Wilhelmus et al., 2006b) and enhance clearance of exogenous Aβ42 in rat hippocampus in vivo (Takata et al., 2003). We demonstrated that the endogenous modest activation of the neuronal stress response was insufficient to prevent Aβ42-induced cell death and that over-expression of Hsp70 reversed Aβ42 toxic effects (Magrane et al., 2004), a result confirmed by others (Zhang et al., 2004). Intraneuronal Aβ42 accumulation was shown to compromise neuron survival by impairing full expression of the stress response. Thus, when inherent cellular protection mechanisms were boosted, Aβ42-induced neuronal death was prevented (Magrane et al., 2005). More recently, over-expression of Hsp70 and Hsp90, together with CHIP, reduced intracellular Aβ levels and, consequently, Aβ-induced neuronal death (Kumar et al., 2007). Additionally, it has been suggested that induction of certain endoplasmic reticulum chaperones can be therapeutically beneficial for the treatment of AD since it decreases Aβ production (Hoshino et al., 2007).

In keeping with the potential for direct increase in Hsp levels, stimulation of HSF1 maybe similarly beneficial. Down-regulation of HSF1 in transgenic worms that over-express intracellular Aβ42 resulted in accelerated paralysis and increased protein aggregation (Cohen et al., 2006). Inhibition of HSF-1 also accelerates aging in wild type (Garigan et al., 2002) and decreases longevity in *c.elegans* in which an age-1 gene mutation in the insulin signaling pathway extends lifespan (Morley and Morimoto, 2004). Although never tested in models of AD, activation of HSF1 and the downstream expression of the stress response has been proven to be therapeutically effective in several neurodegenerative diseases. Thus, the Hsp90 inhibitor geldanamycin activates HSF1 and inhibits α-synuclein aggregation and toxicity both in vitro and in vivo (McLean et al., 2004; Auluck et al., 2005). Furthermore, geldanamycin and radicicol, another Hsp90 inhibitor, suppressed huntingtin aggregation and toxicity in organotypic cultures derived from huntingtin transgenic mice (Hay et al., 2004). Similarly, celastrol, a recently identified drug compound and a component of Chinese herbal medicines, was reported to induce the cellular stress response by activating HSF1 (Westerheide et al., 2004) and to improve memory in normal Sprague-Dawley rats (Allison et al., 2001). Arimoclomol, another HSF1 activator, improves neuronal survival in SOD1 mutant amyotrophic lateral sclerosis mice (Kieran et al., 2004). Acetyl-L-carnitine, a mitochondrial antioxidant, up-regulates Hsp in cortical neurons exposed to Aβ42-oxidative stress (Abdul et al., 2006).

Another relevant Hsp-directed therapeutic strategy would be to remove hyperphosphorylated τ. Reduction of insoluble τ aggregates and τ phosphorylation was achieved in culture by over-expression of Hsp70 and Hsp90, and in vivo by Hsp70 alone (Dou et al., 2003; Petrucelli et al., 2004). Recently, a novel Hsp90 inhibitor promoted selective decreases in phosphorylated τ in a mouse model of tauopathy (Dickey et al., 2007).

Table 1. Heat shock proteins in Alzheimer's disease: Cytoplasmic and mitochondrial chaperone families

Family	Function	AD mechanisms	References
Hsp100: 104–110	ATPase activity. Thermal tolerance, protein disaggregation and refolding. Works with Hsp70	Inhibits Aβ aggregation	Kong et al. (2005)
Hsp90	ATPase activity. Interacts with signal transduction molecules, nuclear hormone receptor maturation, stabilizes misfolded proteins, prevents aggregation of refolded peptides, and ensures correct assembly and folding of newly synthesized client proteins. Hsp90 inhibitors activate HSF1	Associated with Aβ plaques. Binds to APP and τ. Complexes with CHIP. Inhibition of Hsp90 hastens phospho-τ and polyglutamine protein degradation by the proteasome	McLean et al. (2004); Auluck et al. (2005); Zhao and Houry (2005); Dickey et al. (2006); and Waza et al. (2006a)
Hsc70–73 (non-inducible) Hsp70 (stress-inducible): cytoplasmic	ATPase activity. Regulates protein transport and cell cycle. Anti-apoptotic. Binds to, stabilizes and correctly folds nascent polypeptides, refolds denatured proteins, prevents aggregation. Down-regulates HSF1	Binds to APP, Aβ and phospho-τ in complex with CHIP. Reduces levels of phospho-τ, promotes its ubiquitination and increases levels of inert-insoluble τ. Reduces Aβ levels and reverses Aβ and τ toxicities	Kouchi et al. (1999); Fonte et al. (2002); Dou et al. (2003); Magrane et al. (2004); Petrucelli et al. (2004); Shimura et al. (2004b); Zhang et al. (2004); Magrane et al. (2005); and Kumar et al. (2007)
mitochondrial (mortalin)	ATPase activity. Mitochondrial import of proteins and energy production. Interacts with mtHsp60	Unknown	Yaguchi et al. (2007)
Hsp60 (chaperonins) cytoplasmic (TCP1)	Binds to partially folded polypeptides to assist in mature folding (e.g. actin, tubulin), refolds denatured proteins, facilitates degradation	Levels are reduced in Aβ-expression systems.	Zamostiano et al. (1999)
mitochondrial (Group 1)	Facilitates protein folding in the mitochondria. Interacts with mortalin	Protective vs. Aβ toxicity to PDH, αKGDH and electron transport components	Bozner et al. (2002); Veereshwarayya et al. (2006)

(*Continued*)

Hsp40	Co-chaperone to Hsp70, aiding ATP hydrolysis and the closing of the Hsp70 pocket to release folded substrate	No action reported in AD yet, but suppresses inclusion formation in mutant α-synuclein, SOD1 and polyglutamine models	Minami et al. (1996); Jana et al. (2000); McLean et al. (2002); Takeuchi et al. (2002); Klucken et al. (2004); Muchowski and Wacker (2005)
Small Hsp:	ATP independent.		
αB-crystallin	Cytoskeletal stabilization, suppresses aggregation of partial denatured proteins	Interacts with Aβ, APP and ubiquitinated τ, may increase Aβ toxicity	Stege et al. (1999); Liang (2000); Fonte et al. (2002); Narayanan et al. (2006)
Hsp27	Assembles into dynamic oligomers, binds to released cytochrome c and inhibits caspase activation, stabilizes intermediate microfilaments and actin. Suppresses aggregation and heat inactivation of proteins. Cell survival and metabolism homeostasis	Binds phospho-τ, promotes its dephosphorylation and is neuroprotective versus τ toxicity (ubiquitin-independent). Binds Aβ and prevents fibril formation. Increased expression in AD brain. Associated with plaques	Renkawek et al. (1993); Kudva et al. (1997); Stege et al. (1999); Concannon et al. (2003); Shimura et al. (2004)
Hsp20		Binds Aβ and prevents fibril formation, associated with plaques in AD brain.	Lee et al. (2005); Wilhelmus et al. (2006)
HspB2, B8		Suppresses Aβ toxicity in vitro. Associates with vascular Aβ	Wilhelmus et al. (2006)

CONCLUSION

Although still far from application to patients with AD, the sum of these studies provide a rationale for the development of novel therapeutic strategies designed to up-regulate endogenous Hsp levels in order to prevent or reverse protein misfolding and to boost aggregate-clearance mechanisms (see Table 1). Therapeutic stimulation of inducible chaperones like the HSP70 and the small Hsp27 hold promise to restore several proteolytic systems that become overwhelmed in neurodegenerative diseases. Desirable outcomes are to increase ubiquitin-proteasome throughput to reduce levels of soluble toxic proteins, as well as to promote aggresome formation (a centrosome-associated, membrane bound structure to sequester small aggregates) and lysosomal autophagy to clear the bulkier protein aggregates. Given that Hsp are located primarily in intracellular compartments, Hsp induction is likely to protect against Aβ and τ toxicity by binding oligomeric species inside the cell, in

distinction to effects on extracellular aggregates or plaques and intracellular inclusions. This view is supported by some studies that show inclusion body formation per se in α-synuclein and polyglutamine models is not affected by chaperone expression (Warrick et al., 1999; Cummings et al., 2001; Zhou et al., 2001; Auluck et al., 2002). This prediction however deserves to be tested in AD transgenic animals. Moreover, the consequences of possible feedback repression of HSF1 also need to be worked out. Further understanding of the cooperative interactions among molecular chaperones that affect APP processing, intraneuronal Aβ accumulation and abnormal τ phosphorylation would strengthen the chances to translate present knowledge into a practical drug. Delineation of the mechanism behind the impact of the aging process on the stress response will go a long way to develop therapeutic approaches to reverse the toxic effects of aggregated Aβ peptides and τ proteins in AD. Various animal models support the notion that a critical link between aging and the response to misfolded protein stress is mediated by HSF-1 and its HSP transcriptional targets (Hsu et al., 2003; Morley and Morimoto, 2004). The inseparable relationship between AD and aging augers the same benefit.

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